AMENDMENTS

In the Specification:

Please replace the paragraph beginning at page 9, line 19, with the following rewritten paragraph:

B1

--Fig. 21 depicts sequences used in the array (Table 1). Each probe has a 5'-(NH_2-$(CH_2)_6$-) functionality for cyanuric chloride activation and attachment to the microspheres. Each complementary target has a 5'-fluorescein label;--

Please replace the paragraph beginning at page 11, line 31, with the following rewritten paragraph:

B2

--An additional benefit of the present invention is that it allows the synthesis of the bioactive agents (i.e. compounds such as nucleic acids and antibodies) to be separated from their placement on an array, i.e. the bioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface. Since the beads are self-encoded by having dyes present that have known responses to a reference analyte, this means that the array can later be "decoded", i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art. Once the array is loaded with the beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.--

NE = Instructions doesnot correspond

Please replace the paragraph beginning at page 14, line 13, with the following rewritten paragraph:

--Accordingly, the present invention provides array compositions comprising at least a first substrate with a surface comprising individual sites. By "array" herein is meant a plurality of bioactive agents in an array format; the size of the array will depend on the composition and end use of the array. Arrays containing from about 2 different bioactive agents (i.e. different beads) to many millions can be made, with very large fiber optic arrays being possible. Generally, the array will comprise from two to as many as a billion or more, depending on the size of the beads and the substrate, as well as the end use of the array, thus very high density, high density, moderate density, low density and very low density arrays may be made. Preferred ranges for very high density arrays are from about 10,000,000 to about 2,000,000,000, with from about 100,000,000 to about 1,000,000,000 being preferred. High density arrays range about 100,000 to about 10,000,000, with from about 1,000,000 to about 5,000,000 being particularly preferred. Moderate density arrays range from about 10,000 to about 50,000 being particularly preferred, and from about 20,000 to about 30,000 being especially preferred. Low density arrays are generally less than 10,000, with from about 1,000 to about 5,000 being preferred. Very low density arrays are generally less than 1,000, with from about 10 to about 1000 being preferred, and from about 100 to about 500 being particularly preferred. In some embodiments, the compositions of the invention may not be in an array format; that is, for some embodiments, compositions comprising a single bioactive agent may be made as well. In addition, in some arrays, multiple substrates may be used, either of different or identical compositions. Thus for example, large arrays may comprise a plurality of smaller substrates.--

Please replace the paragraph beginning at page 15, line 4, with the following rewritten paragraph:

--The compositions comprise a substrate. By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of beads and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible

substrates are very large, and include, but are not limited to, glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, Teflon™, etc.), polysaccarides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. In general, the substrates allow optical detection and do not appreciably fluorescese.--

Please replace the paragraph beginning at page 16, line 24, with the following rewritten paragraph:

--In a preferred embodiment, the surface of the substrate is modified to contain chemically modified sites, that can be used to attach, either covalently or non-covalently, the microspheres of the invention to the discrete sites or locations on the substrate. "Chemically modified sites" in this context includes, but is not limited to, the addition of a pattern of chemical functional groups including amino groups, carboxy groups, oxo groups and thiol groups, that can be used to covalently attach microspheres, which generally also contain corresponding reactive functional groups; the addition of a pattern of adhesive that can be used to bind the microspheres (either by prior chemical functionalization for the addition of the adhesive or direct addition of the adhesive); the addition of a pattern of charged groups (similar to the chemical functionalities) for the electrostatic attachment of the microspheres, i.e. when the microspheres comprise charged groups opposite to the sites; the addition of a pattern of chemical functional groups that renders the sites differentially hydrophobic or hydrophilic, such that the addition of similarly hydrophobic or hydrophilic microspheres under suitable experimental conditions will result in association of the microspheres to the sites on the basis of hydroaffinity. For example, the use of hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, "pattern" in this sense includes the use of a uniform treatment of the surface to allow attachment of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be

appreciated by those in the art, this may be accomplished in a variety of ways.--

Please replace the paragraph beginning at page 17, line 8, with the following rewritten paragraph:

--The compositions of the invention further comprise a population of microspheres. By "population" herein is meant a plurality of beads as outlined above for arrays. Within the population are separate subpopulations, which can be a single microsphere or multiple identical microspheres. That is, in some embodiments, as is more fully outlined below, the array may contain only a single bead for each bioactive agent; preferred embodiments utilize a plurality of beads of each type.--

Please replace the paragraph beginning at page 17, line 14, with the following rewritten paragraph:

--By "microspheres" or "beads" or "particles" or grammatical equivalents herein is meant small discrete particles. The composition of the beads will vary, depending on the class of bioactive agent and the method of synthesis. Suitable bead compositions include those used in peptide, nucleic acid and organic moiety synthesis, including, but not limited to, plastics, ceramics, glass, polystyrene, methylstyrene, acrylic polymers, paramagnetic materials, thoria sol, carbon graphite, titanium dioxide, latex or cross-linked dextrans such as Sepharose, cellulose, nylon, cross-linked micelles and Teflon™ may all be used.--

Please replace the paragraph beginning at page 22, line 18, with the following rewritten paragraph:

--In a preferred embodiment, the microspheres further comprise a bioactive agent. By "candidate bioactive agent" or "bioactive agent" or "chemical functionality" or "binding ligand" herein is meant as used herein describes any molecule, *e.g.*, protein, oligopeptide, small

organic molecule, polysaccaride, polynucleotide, etc. which can be attached to the microspheres of the invention. It should be understood that the compositions of the invention have two primary uses. In a preferred embodiment, as is more fully outlined below, the compositions are used to detect the presence of a particular target analyte; for example, the presence or absence of a particular nucleotide sequence or a particular protein, such as an enzyme, an antibody or an antigen. In an alternate preferred embodiment, the compositions are used to screen bioactive agents, i.e. drug candidates, for binding to a particular target analyte.--

Please replace the paragraph beginning at page 23, line 13, with the following rewritten paragraph:

--In a preferred embodiment, the bioactive agents are proteins. By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and norleucine are considered amino acids for the purposes of the invention. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradations.--

Please replace the paragraph beginning at page 23, line 30, with the following rewritten paragraph:

--In a preferred embodiment, the bioactive agents are peptides of from about 5 to about 30 amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to about 15 being particularly preferred. The peptides may be digests of naturally occurring

proteins as is outlined above, random peptides, or "biased" random peptides. By "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus forming a library of randomized bioactive proteinaceous agents.--

Please replace the paragraph beginning at page 24, line 27, with the following rewritten paragraph:

--In a preferred embodiment, the bioactive agents are nucleic acids (generally called "probe nucleic acids" or "candidate probes" herein). By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage, *et al.*, Tetrahedron **49**(10):1925 (1993) and references therein; Letsinger, J. Org. Chem., **35**:3800 (1970); Sprinzl, *et al.*, Eur. J. Biochem., **81**:579 (1977); Letsinger, *et al.*, Nucl. Acids Res., **14**:3487 (1986); Sawai, *et al.*, Chem. Lett., 805 (1984); Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); and Pauwels, *et al.*, Chemica Scripta, **26**:141 (1986)), phosphorothioate (Mag, *et al.*, Nucleic Acids Res., **19**:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu, *et al.*, J. Am. Chem. soc., **111**:2321 (1989)) O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem. Soc., **114**:1895 (1992); Meier, *et al.*, Chem. Int. Ed. Engl., **31**:1008 (1992); Nielsen, Nature, **365**:566 (1993); Carlsson, *et al.*, Nature, **380**:207 (1996), all of which are incorporated by reference)). Other analog nucleic acids include those with positive backbones (Denpcy, *et al.*, Proc. Natl. Acad. Sci. USA,

92:6097 (1995)); non-ionic backbones (U.S. Patent Nos. 5,386,023; 5,637,684; 5,602,240; 5,216,141; and 4,469,863; Kiedrowshi, *et al.*, Angew. Chem. Intl. Ed. English, **30**:423 (1991); Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); Letsinger, *et al.*, Nucleosides & Nucleotides, **13**:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, Carbohydrate Modifications in Antisense Research, Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker, *et al.*, Bioorganic & Medicinal Chem. Lett., **4**:395 (1994); Jeffs, *et al.*, J. Biomolecular NMR, **34**:17 (1994); Tetrahedron Lett., **37**:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, Carbohydrate Modifications in Antisense Research, Ed. Y.S. Sanghu and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins, *et al.*, Chem. Soc. Rev., (1995) pp. 169-176). Several nucleic acid analogs are described in Rawls, C & E News, June 2, 1997, page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments. In addition, mixtures of naturally occurring nucleic acids and analogs can be made. Alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made. The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthanine, hypoxanthanine, isocytosine, isoguanine, and basepair analogs such as nitropyrrole and nitroindole, etc.--

Please replace the paragraph beginning at page 25, line 31, with the following rewritten paragraph:

--As described above generally for proteins, nucleic acid bioactive agents may be

naturally occurring nucleic acids, random nucleic acids, or "biased" random nucleic acids. For example, digests of procaryotic or eukaryotic genomes may be used as is outlined above for proteins.--

Please replace the paragraph beginning at page 26, line 1, with the following rewritten paragraph:

--In general, probes of the present invention are designed to be complementary to a target sequence (either the target analyte sequence of the sample or to other probe sequences, as is described herein), such that hybridization of the target and the probes of the present invention occurs. This complementarity need not be perfect; there may be any number of base pair mismatches that will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under the selected reaction conditions. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium).

Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30C for short probes (e.g. 10 to 50 nucleotides) and at least about 60C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, supra, and Tijssen, supra.--

Please replace the paragraph beginning at page 26, line 28, with the following rewritten paragraph:

--The term "target sequence" or grammatical equivalents herein means a nucleic acid sequence on a single strand of nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA and rRNA, or others. It may be any length, with the understanding that longer sequences are more specific. As will be appreciated by those in the art, the complementary target sequence may take many forms. For example, it may be contained within a larger nucleic acid sequence, i.e. all or part of a gene or mRNA, a restriction fragment of a plasmid or genomic DNA, among others. As is outlined more fully below, probes are made to hybridize to target sequences to determine the presence or absence of the target sequence in a sample. Generally speaking, this term will be understood by those skilled in the art.--

Please replace the paragraph beginning at page 31, line 20, with the following rewritten paragraph:

--Fig. 4 illustrates how the microwells 250 are formed and microspheres 10 placed in the microwells. In one embodiment, a 1 mm hexagonally-packed imaging fiber bundle 202 was employed comprising approximately 20,600 individual optical fibers having cores approximately

3.7 μm across (Part No. ET26 from Galileo Fibers, Sturbridge, MA). Typically, the cores of each fiber are hexagonally shaped as a result of glass hardness and drawing during fiber fabrication. In some cases, the shape can be circular, however.--

Please replace the paragraph beginning at page 31, line 27, with the following rewritten paragraph:

--In step 270, both the proximal 214 and distal 212 ends of the fiber bundle 202 are successively polished on 12 μm, 9 μm, 3 μm, 1 μm, and 0.3 μm lapping films. Subsequently, the ends can be inspected for scratches on a conventional atomic force microscope. In step 272, etching is performed on the distal end 212 of the bundle 202. A solution of 0.2 grams NH_4F (ammonium fluoride) with 600 μl dH_2O and 100 μl of HF (hydrofluoric acid), 50% stock solution, may be used. The distal end 212 is etched in this solution for a specified time, preferably approximately 80 seconds.--

Please replace the paragraph beginning at page 32, line 6, with the following rewritten paragraph:

--As illustrated in Figs. 5A and 5B, the foregoing procedure produces microwells by the anisotropic etching of the fiber cores 254 favorably with respect to the cladding 256 for each fiber of the bundle 202. The microwells have approximately the diameter of the cores 254, 3.7 μm. This diameter is selected to be slightly larger than the diameters of the microspheres used, 3.1 μm, in the example. The preferential etching occurs because the pure silica of the cores 254 etches faster in the presence of hydrofluoric acid than the germanium-doped silica claddings 256.--

Please replace the paragraph beginning at page 33, line 22, with the following rewritten paragraph:

--In addition, since the size of the array will be set by the number of unique optical response signatures, it is possible to "reuse" a set of unique optical response signatures to allow for a greater number of test sites. This may be done in several ways; for example, by using a positional coding scheme within an array; different sub-bundles may reuse the set of optical response signatures. Similarly, one embodiment utilizes bead size as a coding modality, thus allowing the reuse of the set of unique optical response signatures for each bead size. Alternatively, sequential partial loading of arrays with beads can also allow the reuse of optical response signatures.--

Please replace the paragraph beginning at page 33, line 30, with the following rewritten paragraph:

--In a preferred embodiment a spatial or positional coding system is done. In this embodiment, there are sub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an "area code", that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique dye/bead combinations can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.--

Please replace the paragraph beginning at page 34, line 19, with the following rewritten paragraph:

--In a preferred embodiment, the coding and decoding is accomplished by sequential loading of the microspheres into an array. As outlined above for spatial coding, in this embodiment, the optical response signatures can be "reused". In this embodiment, the library

of microspheres each comprising a different bioactive agent (or the subpopulations each comprise a different bioactive agent), is divided into a plurality of sublibraries; for example, depending on the size of the desired array and the number of unique tags, 10 sublibraries each comprising roughly 10% of the total library may be made, with each sublibrary comprising roughly the same unique tags. Then, the first sublibrary is added to the fiber optic bundle comprising the wells, and the location of each bioactive agent is determined, using its optical response signature. The second sublibrary is then added, and the location of each optical response signature is again determined. The signal in this case will comprise the first optical response signature and the "second" optical response signature; by comparing the two matrices the location of each bead in each sublibrary can be determined. Similarly, adding the third, fourth, etc., sublibraries sequentially will allow the array to be filled.--

Please replace the paragraph beginning at page 39, line 20, with the following rewritten paragraph:

--The arrays of the present invention are constructed such that information about the identity of the bioactive agent is built into the array, such that the random deposition of the beads on the surface of the substrate can be "decoded" to allow identification of the bioactive agent at all positions. This may be done in a variety of ways.--

Please replace the paragraph beginning at page 40, line 4, with the following rewritten paragraph:

--In a preferred embodiment, the beads are loaded onto the substrate and then the array is decoded, prior to running the assay. This is done by detecting the optical response signature associated with the bead at each site on the array upon exposure to a reference analyte. This may be done all at once, if unique optical signatures are used, or sequentially, as is generally outlined above for the "reuse" of sets of optical signatures. Alternatively, full or partial decoding may occur after the assay is run.--

Please replace the paragraph beginning at page 42, line 6, with the following rewritten paragraph:

--In a preferred embodiment, the compositions are used to probe a sample solution for the presence or absence of a target analyte. By "target analyte" or "analyte" or grammatical equivalents herein is meant any atom, molecule, ion, molecular ion, compound or particle to be either detected or evaluated for binding partners. As will be appreciated by those in the art, a large number of analytes may be used in the present invention; basically, any target analyte can be used which binds a bioactive agent or for which a binding partner (i.e. drug candidate) is sought.--

Please replace the paragraph beginning at page 43, line 25, with the following rewritten paragraph:

--The present invention also finds use as a methodology for the detection of mutations or mismatches in target nucleic acid sequences. For example, recent focus has been on the analysis of the relationship between genetic variation and phenotype by making use of polymorphic DNA markers. Previous work utilized short tandem repeats (STRs) as polymorphic positional markers; however, recent focus is on the use of single nucleotide polymorphisms (SNPs), which occur at an average frequency of more than 1 per kilobase in human genomic DNA. Some SNPs, particularly those in and around coding sequences, are likely to be the direct cause of therapeutically relevant phenotypic variants. There are a number of well known polymorphisms that cause clinically important phenotypes; for example, the apoE2/3/4 variants are associated with different relative risk of Alzheimer's and other diseases (see Cordor et al., Science 261 (1993). Multiplex PCR amplification of SNP loci with subsequent hybridization to oligonucleotide arrays has been shown to be an accurate and reliable method of simultaneously genotyping at least hundreds of SNPs; see Wang et al., Science, 280:1077 (1998); see also Schafer et al., Nature Biotechnology 16:33-39 (1998). The compositions of the present invention may easily be substituted for the arrays of the prior art.--

Please replace the paragraph beginning at page 44, line 12, with the following rewritten paragraph:

--In a preferred embodiment, the binding of the bioactive agent and the target analyte is specific; that is, the bioactive agent specifically binds to the target analyte. By "specifically bind" herein is meant that the agent binds the analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. However, as will be appreciated by those in the art, it will be possible to detect analytes using binding which is not highly specific; for example, the systems may use different binding ligands, for example an array of different ligands, and detection of any particular analyte is via its "signature" of binding to a panel of binding ligands, similar to the manner in which "electronic noses" work. This finds particular utility in the detection of chemical analytes. The binding should be sufficient to remain bound under the conditions of the assay, including wash steps to remove non-specific binding, although in some embodiments, wash steps are not desired; i.e. for detecting low affinity binding partners. In some embodiments, for example in the detection of certain biomolecules, the dissociation constants of the analyte to the binding ligand will be less than about 10^{-4} -10^{-6} M^{-1}, with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} -10^{-9} M^{-1} being particularly preferred.--

Please replace the paragraph beginning at page 47, line 21, with the following rewritten paragraph:

--**Data analysis:** In the data analysis portion, pre-selected segments taken from a previously collected "focus" image are transferred to the sequence of images collected. These segments, drawn by the user, allow the mean pixel intensity to be measured in particular regions throughout the image field. Typically, they are drawn over individual pixels of a fiber optic sensor array, each of which contains a bead. The script then enters a loop that steps through each frame, measuring the mean pixel intensity within each segment, and placing the values in data columns. The resulting columns can then be plotted to yield the temporal

response of each bead of interest. Before plotting, however, responses are "standardized" by dividing the data for each bead response by its first point. Thus, all responses can be normalized to start at a value of 1.0.--

Please replace the paragraph beginning at page 47, line 31, with the following rewritten paragraph:

--**Redundancy:** As shown in the Examples, the present invention shows that building redundancy into an array gives several significant advantages, including the ability to make quantitative estimates of confidence about the data and significant increases in sensitivity. Thus, preferred embodiments utilize array redundancy. As will be appreciated by those in the art, there are at least two types of redundancy that can be built into an array: the use of multiple identical sensor elements (termed herein "sensor redundancy"), and the use of multiple sensor elements directed to the same target analyte, but comprising different chemical functionalities (termed herein "target redundancy"). For example, for the detection of nucleic acids, sensor redundancy utilizes of a plurality of sensor elements such as beads comprising identical binding ligands such as probes. Target redundancy utilizes sensor elements with different probes to the same target: one probe may span the first 25 bases of the target, a second probe may span the second 25 bases of the target, etc. By building in either or both of these types of redundancy into an array, significant benefits are obtained. For example, a variety of statistical mathematical analyses may be done.--

Please replace the paragraph beginning at page 52, line 5, with the following rewritten paragraph:

--In this embodiment, a plurality of different sensor elements may be used, with from about 2 to about 20 being preferred, and from about 2 to about 10 being especially preferred, and from 2 to about 5 being particularly preferred, including 2, 3, 4 or 5. However, as above, more may also be used, depending on the application.--

Please replace the paragraph beginning at page 52, line 12, with the following rewritten paragraph:

--One benefit of the sensor element summing (referred to herein as "bead summing" when beads are used), is the increase in sensitivity that can occur. As shown in Example 19, detection limits in the zeptomole range can be observed.--

Please replace the paragraph beginning at page 59, line 5, with the following rewritten paragraph:

--Before attaching oligonucleotides to the microspheres, a family of dye-encoded microspheres was created. Fluorescent dyes were used to encode the microspheres. Europium(III)thenoyltrifluoro-acetonate-$3H_2O$ ($_{ex}/_{em}$=365/615) (Eu-dye), ($_{ex}/_{em}$=620/700) and 5-(and -6)-carboxytetramethyl-rhodamine, succinimidyl ester ($_{ex}/_{em}$=535/580) (TAMRA, SE) were chosen for this demonstration. The dyes were incorporated by exploiting the chemical properties of the amino-modified polystyrene microspheres as follows. 200-µL-aliquots of stock (1 mL of stock beads contains $5.8x10^9$ beads in 0.01% merthiolate in water) 3.1 µm-diameter amine-modified poly(methylstyrene)divinylbenzene microspheres (Bangs Laboratories, Inc. Carmel, IN) were filtered and washed with dry THF then placed in a microcentrifuge tube. 200 µL of europium(III)thenoyltrifluoroacetonate-$3H_2O$ [Eu-dye (Acros)] dye in THF was added to the beads. Eu-dye concentrations of 0, 0.001, 0.01, 0.025, 0.05, 0.1, 0.5, and 1 M were used. The microsphere/dye suspension was shaken (VWR Vortex Genie II) for 2 h. The suspensions were filtered separately (Millipore Type HVLP) and washed thoroughly with MeOH. The beads were stored in 0.0% Tween (essential for preparation and storage to prevent the beads from clumping together) in ultrapure water until use.--

Please replace the paragraph beginning at page 59, line 20, with the following rewritten paragraph:

--Alternatively, external encoding was done. 10 µL of stock beads were rinsed (all rinsing procedure entailed placing the centrifuge tube containing the beads and solution into a microcentrifuge at 8000 rpm for 3 min, and liquid over the beads was removed using a pipette) with BT buffer (0.1 M boric acid, 0.1 M NaOH, 0.13 M $NaHCO_3$, 0.01% Tween, pH 9). The beads were suspended in 100 µL BT buffer then 5 µL of dye solution [Cy5 (Amersham) or TAMRA(Molecular Probes)] in DMF was added. Cy5 concentrations of 0, 0.01, 0.05, 0.1, 0.3 mM and TAMRA concentrations of 0, 0.1, 0.4, and 3 mM were used. The beads were shaken for 2 h then rinsed three times with BT buffer then three times with PBST buffer (0.01 M phosphate buffer saline, 0.0% Tween, pH 7.4).--

Please replace the paragraph beginning at page 59, line 29, with the following rewritten paragraph:

--The polystyrene microspheres swell in tetrahydrofuran (THF) enabling a dye to penetrate the microsphere and become entrapped with the microsphere contracts. The absorption and emission spectra of the dyes are not compromised within the microsphere's environment and their concentration remains constant over time. Eight distinguishable microsphere families were prepared by entrapping varying Eu-dye concentrations inside the microspheres. In addition to internal entrapment, the microspheres' amine-modified surface permitted coupling to amine-reactive dyes. Different concentrations of Cy5 and TAMRA were then attached to the surface amine groups of the eight Eu-dye beads. A library of 100 spectroscopically-distinguishable microsphere types was prepared using various combinations of the three dyes. Microsphere encoding was carried out prior to oligonucleotide attachment because reaction with the amine reactive dyes after probe attachment affected the hybridization reaction. On the other hand, the oligonucleotide probes on the surface of the microspheres are not affected by subsequent internal encoding with Eu-dye.--

Please replace the paragraph beginning at page 60, line 13, with the following rewritten paragraph:

--DNA probes were synthesized with a 5'-amino-C6 modifier (Glen Research) in the Tufts Physiology Department using an ABI synthesizer. 20 nmol of the 5'-amino-terminal oligonucleotide probe were dissolved in 180 µL of 0.1 M sodium borate buffer (SBB pH 8.3). Oligonucleotide activation was initiated by adding 40 nmol of cyanuric chloride in 40 µL of acetonitrile. After 1 h, unreacted cyanuric chloride was removed by three cycles of centrifugal ultrafiltration (Microcon 3, Amicon) and recovered in 200 µL of 0.1 M SBB.--

Please replace the paragraph beginning at page 60, line 20, with the following rewritten paragraph:

--**DNA functionalization.** Five µL of stock beads were rinsed with 0.02 M phosphate buffer (pH 7). 150 µL of 5% glutaraldehyde in phosphate buffer was added to the beads. The beads were shaken for 1 h then rinsed three times with phosphate buffer. 150 µL of 5% polyethyleneimine (PEI) was then added to the beads. The beads were shaken for 1 h then rinsed three times with phosphate buffer then three times with 0.1 M SBB (sodium borate buffer, pH 8.3). 100 µL of 150 µM cyanuric chloride-activated oligonucleotide probe in SBB buffer was added to the beads and shaken overnight. The probe solution was removed and saved to reuse. The beads were then rinsed three times with SBB buffer. Remaining amine groups were capped with succinic anhydride to prevent non-specific binding. 100 µL of 0.1 M succinic anhydride in 90% DMSO, 10% SBB was added to the beads. The beads were shaken for 1 h then rinsed three times with SBB buffer then three times with TE buffer (10 mM Tris-HCL, pH 8.3, 1 mM EDTA, 0.1 M NaCl, 0.1% SDS).--

Please replace the paragraph beginning at page 61, line 7, with the following rewritten paragraph:

--**Microsphere-based fiber-optic sensors.** Recently, we reported an array consisting of randomly distributed independently addressable micron-bead-sensors using an imaging-optical-fiber substrate. This system employed imaging fibers consisting of six thousand

individually clad fibers that were melted and drawn together to form a coherent, 500-µm diameter bundle. The compositional difference between the core and cladding of each fiber enables the cores to be etched selectively providing for the simultaneous formation of six thousand 3.5 µm-diameter wells in the surface of the fiber tip within seconds. See Michael et al., Anal. Chem 70:1242 (1998); Bronk et al., Anal. Chem. 67:2750 (1995) and Pantano et al., Chem. Materials 8:2832 (1996), all of which are incorporated by reference.--

Please replace the paragraph beginning at page 61, line 16, with the following rewritten paragraph:

--**Microwell formation.** 500 µm-diameter imaging fiber bundles containing $6x10^4$ individual fibers were chemically etched according to a previously detailed procedure; see Pantano et al. Chem. Materials 8:2832 (1996).

Please replace the paragraph beginning at page 61, line 20, with the following rewritten paragraph:

--**Array formation.** Five µL of probe-functionalized beads were stored in 40 µL of TE buffer. After selecting the desired probe-functionalized microspheres, 1 µL of each bead solution was placed in a microcentrifuge tube and vortexed. 0.05 µL of this mixture was placed onto the distal face of the imaging fiber containing the microwells. After evaporation of the solvent (approximately 3 min), the distal tip of the fiber is wiped with an anti-static swab to remove excess beads. When a new sensor is desired, sonicating the fiber tip for 3 min will regenerate the substrate.--

Please replace the paragraph beginning at page 61, line 30, with the following rewritten paragraph:

--**Controlling array formation.** One of the primary advantages of this system is the

ability to alter the types of microspheres contained in an array. Each milliliter of stock solution contains approximately $6x10^9$ microspheres enabling functionalization of billions of beads at once. Even after a 20x dilution a 1 µL volume of microsphere solution contains enough beads to produce hundreds of different arrays. The density of microspheres in solution can control th number of occupied wells. With dilute solutions, empty wells remain after the initial array production. Additional microspheres bearing different probes can be added to the unoccupied sites or to the original solution at any time to create a more diverse array. If a different selection of beads is desired, sonicating the fiber tip removes all of the beads from the wells, enabling a new sensor array to be made in the same substrate.--

Please replace the paragraph beginning at page 62, line 22, with the following rewritten paragraph:

--The fiber was not removed from the imaging system during testing, rinsing, or regeneration steps. The proximal tip of the fiber was secured in the fiber chuck of the imaging system and all solutions were brought to the fiber's distal tip which housed the microbead sensors. Images acquired immediately prior to each test while the fiber tip was in buffer were subtracted from the response images. Background signals from empty wells were then subtracted from signals generated during each test.--

Please replace the paragraph beginning at page 62, line 28, with the following rewritten paragraph:

--**Hybridization in real time.** Each microsphere's fixed position made possible a hybridization study in real time. A DNA array containing identical beads was placed on the imaging system. The distal tip of the fiber bearing the microsphere sensors was placed in a labeled-target solution. Emission from hybridizing labeled-target was captured every minute for several minutes. In the small region of the imaging fiber selected for this study, 70 microspheres held the probe complementary to the target in solution. Each microsphere was

monitored independently and simultaneously. Signals from 40 beads were averaged to provide kinetic data. At relatively high concentrations of target, hybridization could be detected immediately, as seen by the steep slope of the data. While the sensor remained on the imaging system it was regenerated by dipping the fiber tip into a room-temperature formamide solution. The same microspheres were assayed several times by placing the regenerated fiber into the target solution and repeating the experiment. Consecutive studies show that the same sensor can be used for multiple tests.--

Please replace the paragraph beginning at page 63, line 6, with the following rewritten paragraph:

--A background fluorescence image was acquired at wavelengths specific to fluorescein (excitation 490 nm emission 530 nm) with the fiber's distal tip in buffer. The fiber's distal tip was then placed in 4 µL of fluorescein-labeled target solution and one image was acquired every minute for 10 min. Subsequently, the fiber was dipped in 90% formamide in TE buffer at room temperature (rt) to regenerate the sensor and a background image was taken with the fiber in buffer. The fiber was again placed in the target solution where images were acquired for another 10 min interval.--

Please replace the paragraph beginning at page 63, line 23, with the following rewritten paragraph:

--The fiber's distal tip was placed in 4 µL of labeled-target solution for 5 min, rinsed with TE buffer, and a fluorescence image was acquired for 5 s. The fiber tip was then dipped in 90% formamide in TE (rt) to remove any hybridized target and regenerate the sensor. This procedure was repeated 100 times using the IL2 target and 5 times (intermittently during the IL2 tests) using the IL6 target.--

Please replace the paragraph beginning at page 64, line 1, with the following rewritten

paragraph:

--**Microsphere sensitivity.** The fiber's distal tip was placed in 4 µL of target solution until the hybridization signal to noise ratio was three. The signal was monitored after rinsing the fiber tip with TE buffer and acquiring a fluorescence image for 5 s while the fiber tip was in buffer. For the hour-long assays, a 0.6 mL centrifuge tube was filled and capped. A hole was drilled in the cap to enable the fiber tip to be placed in the target solution while preventing evaporation.--

Please replace the paragraph beginning at page 64, line 7, with the following rewritten paragraph:

--**Sensitivity with an intensified CCD camera.** The 21-mer cystic fibrosis oligonucleotide probe and complement with F508C mutation (5'-TAT CAT CT**G** TGG TGT TTC CTA-3') were used for this study. The 5'-amino-terminal oligonucleotide probe was activated with 100 times excess of cyanuric chloride. The microspheres were incubated with 400 M cyanuric chloride-activated oligonucleotide. The fluorescein-labeled target was dissolved in 6X saline sodium phosphate EDTA buffer (SSPE) containing 0.1% SDS. The fiber's distal tip was placed in 10 µL of target solution during hybridization with occasional stirring. The distal tip was then washed with 6X SSPE and a fluorescence image was acquired with a Pentamax ICCD camera (Princeton Instruments) for 1 s while the fiber tip was in 120 µL of 6X SSPE.--

Please replace the paragraph beginning at page 64, line 30, with the following rewritten paragraph:

--The fiber's distal tip was placed in a target solution for 5 min, rinsed with TE buffer, and fluorescence images were acquired for 5 s while the fiber was in buffer. Overlay segments were drawn to select the beads bearing a hybridization signal using IPLab software. These overlay segments were copies and pasted onto each of the encoding images and the selected

beads' identity was determined. The sensor was regenerated as described above and this procedure was repeated for each of the target solutions.--

Please replace the paragraph beginning at page 65, line 1, with the following rewritten paragraph:

--**Hybridization specificity in a multiplex assay.** To demonstrate this microsphere array system, we first selected seven probes used in previous work (sequences 1-7 of Table 1, see figure 22). The DNA sequences chosen for the array were designed to be completely specific at room temperature. The signals at two of the three encoding wavelengths is used to positionally register the microspheres. After registration at the encoding wavelengths, the array is ready for use. The fiber tip is dipped into a fluorescent-labeled target solution. After a specified time, the fiber tip is removed from the target solution, rinsed with buffer, and placed in buffer solution. Microspheres bearing a complementary probe display a fluorescent signal due to the hybridized labeled target. Completely specific hybridizations for seven different targets in an array were observed. Replicates of each bead type located randomly within the array yield redundant information which contributes to the array's reliability. Table 2 (Figure 23 shows the accuracy of the system to correctly identify the target.--

Please replace the paragraph beginning at page 66, line 1, with the following rewritten paragraph:

--**Sensitivity of the microspheres.** There are three aspects to sensitivity: sample volume, target concentration, and absolute number of target molecules. The smaller the volume required, the less a sample needs to be amplified for detection since the same number of absolute target molecules in a smaller volume generates a higher local concentration. Sample volumes as small as 4 μL are required with this system since only the tip of the 500 μm-diameter fiber is dipped into the solution. Typically, we use 10 μL volumes for easier handling and to avoid evaporation.--

Please replace the paragraph beginning at page 66, line 17, with the following rewritten paragraph:

--Sensitivity experiments were carried out as follows: the array was hybridized in 10 µL solutions containing progressively decreasing concentrations of labeled target. The lowest concentration evaluated was 1 fM. At various times, the array was taken out of the hybridization solution, rinsed, and a fluorescence image was collected. The array was then placed back into the hybridization buffer. After hybridization, the array was dehybridized with formamide and five background measurements were taken in 6X SSPE. ROI's from 10 or 100 beads in the five images were averaged to provide the mean background. The mean background values were subtracted from the fluorescence intensities of the various numbers of beads. Individual beads exhibited significant variability such that it was not possible to ascertain whether or not a signal was present. On the other hand, summing signals from multiple beads provided detectable signals. The average signal of ten beads gave a 7% CV while 100 beads provided more precise average values with 3% CV. Results from three representative sets of ten beads for the complementary target and two non-complementary targets are presented in Table 5. The hybridization time was determined when the signal was over three times the standard deviation of the background signals (>3sd). Using this criterion, the microsphere-fiber-optic system is able to detect a 1 fM target solution using a 10 µL volume in 1 hour.--

Please replace the paragraph beginning at page 66, line 32, with the following rewritten paragraph:

--Both 10 and 100 beads from a total of 500 beads in the array were selected and monitored. In a 1 fM target solution, 10 µL contains ca. 6000 DNA molecules. With 500 identical beads in the array giving a signal, each bead would be expected to contain, on average, ca. 12 labeled target molecules on its surface. To confidently attest to the generation of signal, the average signal of at least ten beads was needed. Therefore, this system can give

sufficient signal with only 120 molecules.--

Please replace the paragraph beginning at page 67, line 17, with the following rewritten paragraph:

--Since fluorescein was used to label the DNA targets, we selected encoding dyes with spectral properties that would not overlap with the fluorescein spectrum. Covalently binding these dyes to the surface of the amine-functionalized microspehres yielded stable and reproducible signals. Unfortunately, such surface encoding reduces the number of amines available for the cyanuric chloride-activated oligonucleotide probe. Therefore, the concentrations of the dyes were optimized to enable sufficient signals from both the encoding dyes and the hybridized target. The finite number of surface amine groups reduces the range and number of dye combinations that can be generated with an external-labeling scheme. To increase the number of encoded microspheres, dyes also can be entrapped inside the bead. Lanthanide dyes are suitable for such internal encoding. The dyes' spectra are not compromised and their intensity remains constant once inside the microsphere.--

Please replace the paragraph beginning at page 68, line 24, with the following rewritten paragraph:

--The DNA microarray presented here has smaller feature sizes and higher packing densities compared to other DNA arrays. We have demonstrated the fiber optic microarray using a 500 μm-diameter imaging fiber with well diameters of 3.5 m. Fibers have also been tapered to produce nanometer scale wells serving as host to nanometer-diameter beads. Using longer fibers, the microarray sensor tp can be brought to the sample and used to sequentially test multiple solutions. Utilizing the imaging fiber's remote sensing capabilities, arrays with nanometer dimensions potentially can be used for direct intracellular analysis.--